Occurrence of methyl esters in the pancreas

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SUMMARY By column chromatography two lipid fractions, x_1 and x_2, of which there are only traces in the lipids of other organs, have been isolated from the pancreatic lipids. The lipid fraction x_2, which forms a very considerable proportion of the total lipids of the pancreas, has been found to be composed of the methyl esters of lauric, myristic, tetradecenoic, palmitic, palmitoleic, stearic, oleic, linoleic, and arachidic acids. Fraction x_1 was not analyzed.

KEY WORDS methyl esters · pancreas · man · dog · thin-layer chromatography · alumina

IN HER EXTENSIVE INVESTIGATION of the lipids in human organs and tissues, Nieminen[1] found that the pancreas contained two unexpected lipid fractions (x_1 and x_2). Their quantity was remarkably large in the pancreas as compared with other tissues. The aim of the present investigation was to study the composition of x_2.

MATERIALS AND METHODS

Human pancreases were obtained by autopsy at the Forensic Institute of Helsinki University and at the Patho-anatomical Department of Kivelä Hospital. The cadavers had been placed in a freezing room as soon as possible to prevent autolysis. Dog pancreases were obtained from 7-month-old animals. The dogs were autopsied immediately after killing.

All the solvents were of analytical grade.

Extraction of Lipids

Immediately after the autopsy the pancreas was cut into sections 20–30 μ thick using a freezing microtome, and the samples were extracted three times with chloroform–methanol 2:1 in an Erlenmeyer flask. Each time, the sample was extracted with a volume of solvent about 10 times the volume of the tissue for a period of 1 hr. The combined extracts were washed in a separatory funnel with one-fifth of their total volume of distilled water. The chloroform layer was evaporated to dryness in a water bath at 60°, and the residue was dried over silica gel.

Column Chromatography of Lipids

Aluminum oxide (prepared according to Brockmann's procedure, from E. Merck AG), 19 g, was suspended in petroleum ether (bp 40–60°, British Drug Houses Ltd., Poole, Dorset, England) and transferred to the column. The dimensions of the chromatographic column were 350 × 10 mm. The petroleum ether was allowed to flow through the column and discarded. The human pancreatic lipids (305 mg) were dissolved in 3 ml of petroleum ether and added to the column. Elution was carried out at a rate of 1 ml/min. The fractions emerging from the column were evaporated to dryness and the residues weighed. The eluted lipids were examined using TLC on silicic acid.

Thin-layer Chromatography

The TLC plates were prepared by applying a slurry of 30 g of Silica Gel G (Kieselgel G according to Stahl, E. Merck AG) in 60 ml of water. The plates were activated at 110° for 2 hr and stored in a desiccator over silica gel. When neutral lipids were to be examined, plates were developed first with *n*-hexane, dried for 30 min, and further developed with *n*-hexane–benzene 5:95. Chloroform was used for examining the steroids, and chloroform–methanol–water 24:7:1 for the mono- and diglycerides, phospholipids, and free fatty acids. The plates were dried in air, sprayed with ammonium molybdate–perchloric acid reagent (1) and heated at 110° for 15 min to develop the spots, which turned dark blue or brown and were clearly outlined on the white background.

TLC of fraction x_2 was carried out on a layer of silica gel impregnated with silver nitrate. The plates were

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

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[1] E. Nieminen, unpublished experiments.

prepared by the method of De Vries and Jurriens (2): 70 g of Silica Gel G was suspended in a solution containing 30 g of analytical grade silver nitrate in 140 ml of distilled water, and the suspension was spread on glass plates to give a layer 0.25 mm thick. The plates were allowed to dry in the dark room at room temperature for 1 hr and then activated by heating for 1 hr at 110°, and developed with *n*-hexane–ether 85:15. The plates were sprayed after elution with a 0.2% solution of 2,7-dichlorofluorescein in ethanol. The spots appeared yellow on a violet background when the plate was irradiated with ultraviolet light.

Gas–Liquid Chromatography

A Perkin-Elmer 116 gas–liquid chromatograph was used to separate and identify the fatty acid esters. A Perkin-Elmer "BDS" column 1 m in length was used; the column temperature was 180°; helium was the carrier gas; and a hot wire detector was employed.

Infrared Analysis

The infrared spectra were obtained with a Beckman IR-4 double-beam infrared spectrophotometer equipped with sodium chloride prisms. The scans were made with the samples pressed into potassium bromide pellets or between NaCl plates.

TABLE 1 FRACTIONATION OF PANCREATIC LIPIDS (305 MG) ON AN ALUMINA COLUMN

Effluent Fraction Number	Eluent	Volume	Lipid Group	Amount Isolated
		ml		*mg*
1	Petroleum ether	20	Hydrocarbons	—
2	Petroleum ether–ethyl ether 99.5:0.5	200	Fraction x_2	55.6
3	Petroleum ether–ethyl ether 99.3:0.7	100	—	—
4	Petroleum ether–ethyl ether 99.0:1.0	200	Sterol esters	1.9
5	Petroleum ether–ethyl ether 97.0:3.0	100	Fraction x_1	1.1
6	Petroleum ether–ethyl ether 50:50	100	Triglycerides	36.8
7	Chloroform	100	Sterols	34.2
8	Chloroform–methanol 50:50	100	Mono- and diglycerides and phospholipids	61.9
9	Ethanol–chloroform–water 5:2:2	100	Free fatty acids and traces of phospholipids	109.6
			Total	301.1

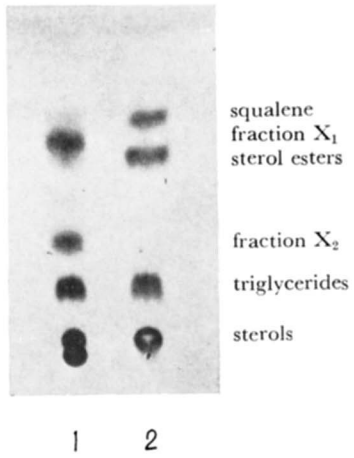

FIG. 1. TLC of human pancreatic lipid extract on silica gel *1*, extract from 10 mg of fresh tissue; *2*, reference compounds: squalene 5 μg, cholesteryl palmitate 5 μg, tristearin 50 μg, cholesterol 5 μg. Developed with (*a*) *n*-hexane, 10 cm; (*b*) *n*-hexane–benzene 5:95, 8 cm. Detection method: ammonium molybdate–perchloric acid spray.

Alkaline Hydrolysis of Fraction x_2

Fraction x_2 (27 mg) was saponified with 0.5 N ethanolic potassium hydroxide (1.5 ml) by boiling the mixture for 1 hr under reflux. Water, 4 ml, was added to the cooled mixture and about 1 ml was distilled from the mixture. Methanol was detected in the distillate by means of its reaction with chromotropic acid (3). The fatty acids were extracted three times with ether from the saponification residue after acidification with hydrochloric acid. The combined ether extracts were washed with water, and the residue was evaporated to dryness and weighed. An IR spectrum of the residue was scanned.

RESULTS AND DISCUSSION

Figure 1 shows a thin-layer chromatogram of the lipids of the human pancreas extracted with chloroform–methanol. Fraction x_1 lies between the squalene and sterol ester spots, and fraction x_2 between the sterol ester and triglyceride spots. The sterols have risen slightly above the starting point, the more polar lipids remaining at the starting point.

The fractionation of human pancreatic lipids on an alumina column is presented in Table 1. From this adsorbent fraction x_2 is eluted immediately after the paraffin hydrocarbons. Thereafter the sterol esters and fraction x_1 are eluted. Elution with petroleum ether–ether in a silicic acid column following the method of Hirsch and Ahrens (4) led to the following elution order: hydrocarbons, sterol esters, fraction x_1, fraction x_2. The separation was not as sharp, however, as with alumina: the sterol esters and fractions x_1 and x_2 overlapped to some extent.

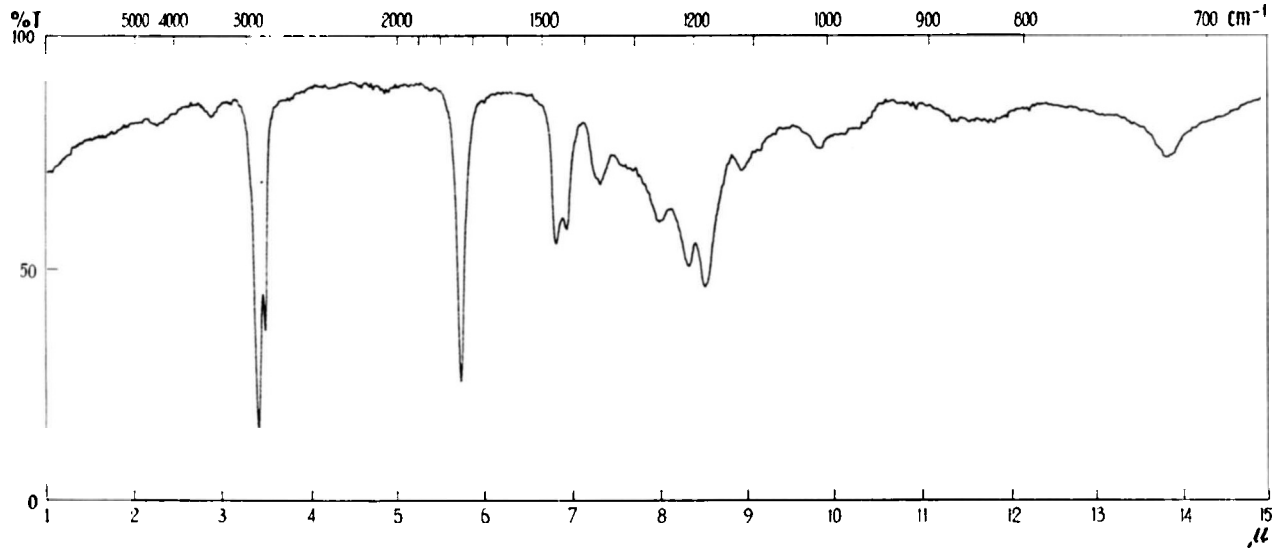

FIG. 2. Infrared spectrum of fraction x_2, prepared between NaCl plates.

From the infrared spectrum of fraction x_2 (Fig. 2) it was concluded that the fraction consisted of an aliphatic, long-chain, carboxylic acid ester, probably methyl ester. The results of elementary analysis (C 76.11%, H 12.12%, O 11.91%) and the molecular weight (260), as determined by the Rast method, were within the range expected for a mixture of methyl esters. When fraction x_2 (27 mg) was saponified, methanol (detected by chromotropic acid) and fatty acids (24 mg) were produced.

Figure 3 shows chromatograms, developed on plates impregnated with silver nitrate, of four different x_2 fractions isolated from the human pancreas. The fraction was resolved into five subfractions. The highest R_F value was the same as that of methyl palmitate, the second highest equalled that of the methyl esters of palmitoleic and oleic acids, and the third highest the same as that of methyl linoleate. No attempts were made to identify the spots that remained on the starting line or moved only a short distance.

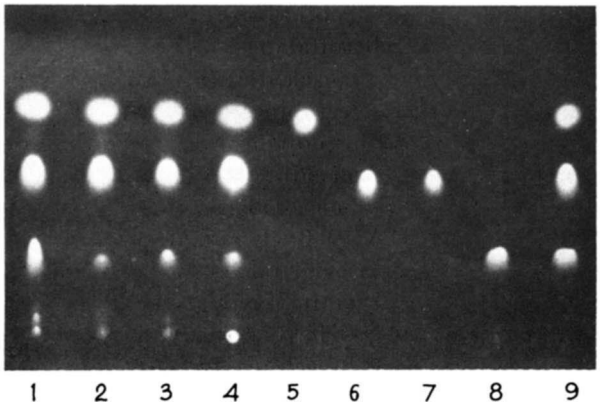

FIG. 3. TLC of fraction x_2 on silica gel impregnated with silver nitrate. *1–4*, fractions x_2 from four different pancreases, 100 μg of each; *5*, methyl palmitate, 20 μg; *6*, methyl palmitoleate, 20 μg; *7*, methyl oleate, 20 μg; *8*, methyl linoleate, 20 μg; *9*, mixture of last four esters, 20 μg of each. Developed with *n*-hexane–ethyl ether 85:15, 10 cm. Detection method: 2, 7-dichlorofluorescein spray.

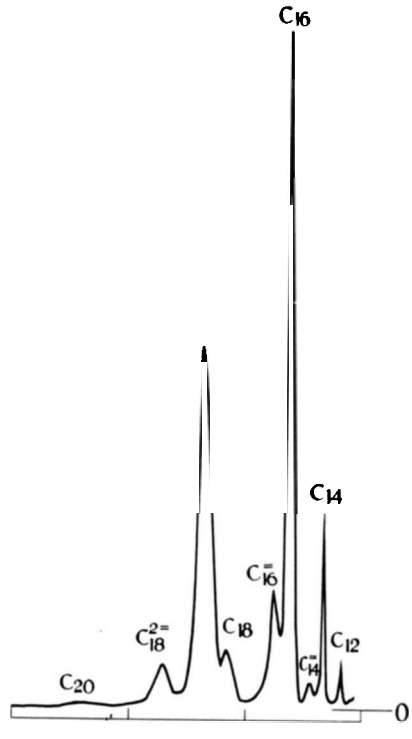

FIG. 4. GLC of fraction x_2. Peaks correspond to methyl esters as shown, where = denotes one double bond.

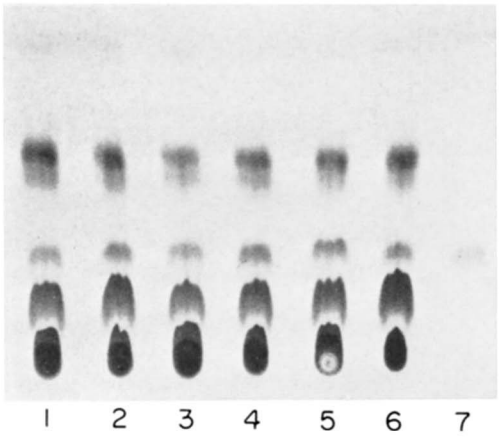

FIG. 5. TLC of human pancreatic lipids extracted with the following solvents: *1*, acetone–chloroform 1:2; *2*, chloroform; *3*, glycerol–chloroform 1:9; *4*, propylene glycol–chloroform 1:9; *5*, chloroform–methanol 99:1; *6*, chloroform–methanol 2:1. The lipids correspond to 10 mg of fresh tissue. *7*, methyl oleate, 20 μg. The developing solvents and detection method are the same as in Fig. 1.

Fraction x_2 was also analyzed by gas chromatography. It was concluded from the retention times of the resulting subfractions that this fraction contained the methyl esters of lauric, myristic, tetradecenoic, palmitic, palmitoleic, stearic, oleic, linoleic, and arachidic acids, although only traces of the methyl esters of the last-mentioned were present (Fig. 4).

Small quantities of the methyl esters of fatty acids have been found in the lipids of animal and human tissues in earlier investigations (5–7). These have been assumed to arise either by esterification or by alcoholysis during extraction with a solvent containing methanol (5). Figure 5 shows thin-layer chromatograms of the lipids extracted from specimens taken from a human pancreas using acetone–chloroform 1:2, chloroform, glycerol–chloroform 1:9, propylene glycol–chloroform 1:9, and two chloroform–methanol mixtures (99:1, 2:1). In all the chromatograms the fractions containing the methyl esters of fatty acids are very distinct. It can thus be concluded that the methyl esters were not formed from the methanol during the extraction.

These results agree with the observations made by Dhopeshwarkar and Mead (6) and Kaufmann and Viswanathan (7). These researchers have found that small quantities of the methyl esters of fatty acids are contained not only in the chloroform–methanol extract but also in chloroform–propanol and ether (6) and petroleum ether (7) extracts of some animal and human tissues.

To confirm that the methyl esters are not artifacts produced post mortem, lipids were extracted from the pancreases of six dogs 5 to 10 min after killing. Figure 6 shows thin-layer chromatograms of the lipids extracted from dog pancreas using chloroform–methanol. The spot indicating the methyl esters of the fatty acids is very distinct in these chromatograms as well.

It should be noted that the methyl esters form a very considerable proportion of the total lipids in the pancreas.

Manuscript received November 27, 1964; accepted June 7, 1965.

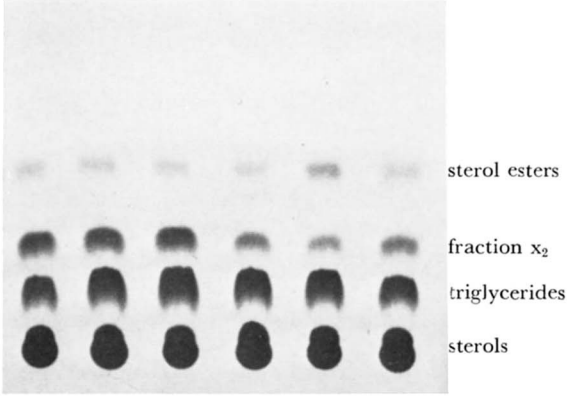

FIG. 6. TLC of dog pancreas lipids. The lipids correspond to 10 mg of fresh tissue. The developing solvents and detection method are the same as in Fig. 1.

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